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Sirolimus Therapy for Angiomyolipoma in Tuberous Sclerosis and Sporadic Lymphangioleiomyomatosis: A Phase 2 trial

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Translational Relevance

Inhibition of the mTOR complex 1 (mTORC1) is important in the treatment of a diverse and growing range of tumours. However, in most cases the tumours concerned are highly heterogeneous at a molecular genetic level and tumour responses are unpredictable. By contrast, renal angiomyolipomas that develop in patients with the inherited disorder tuberous sclerosis and the related sporadic disorder lymphangioleiomyomatosis are specifically associated with inactivation of the *TSC1* or *TSC2* tumour suppressor genes that are key regulators of mTORC1. In this phase II trial 16 patients with these disorders and renal angiomyolipomas were treated for 2 years with the mTORC1 inhibitor sirolimus. Angiomyolipoma burden was reduced in all patients and a response by RECIST criteria was observed in 50% overall and in 80% of the per protocol group. Our results suggest that mTORC1 inhibition may be an effective strategy for treating angiomyolipoma in these rare disorders and merits further study.

Abstract

PURPOSE

Renal angiomyolipomas are a frequent manifestation of tuberous sclerosis and sporadic lymphangioleiomyomatosis. These disorders are associated with mutations of *TSC1* or *TSC2* that lead to over-activation of mammalian target of rapamycin complex 1 (mTORC1), suggesting an opportunity for targeted therapy using mTORC1 inhibitors. This study investigated the efficacy and safety of the mTORC1 inhibitor sirolimus for treatment of renal angiomyolipomas in patients with these disorders.

EXPERIMENTAL DESIGN

In this multicenter phase 2 non-randomized open label trial sixteen patients with tuberous sclerosis or sporadic lymphangioleiomyomatosis and renal angiomyolipoma(s) were treated with oral sirolimus for up to 2 years. Steady state blood levels were 3-10ng/ml. The primary outcome was change in size of renal angiomyolipomas measured by magnetic resonance imaging and assessed by RECIST criteria. Secondary outcomes included safety, neurocognitive function and pulmonary function.

RESULTS

The response rate, by RECIST criteria, was 50%. Summated angiomyolipoma diameters were reduced in all 16 patients and by 30% or more in 8 (all from the per-protocol group of 10). Forty one of 48 angiomyolipomas were smaller at the last measurement than at baseline. Most shrinkage occurred during the first year of treatment. There was little change in pulmonary function. Recall memory improved in 7 of 8 patients with tuberous sclerosis. Adverse events were consistent with the known toxicities of sirolimus.

CONCLUSIONS

This study demonstrated sustained regression of renal angiomyolipomas in patients with tuberous sclerosis or sporadic lymphangioleiomyomatosis receiving 2 years of sirolimus treatment. Possible effects on pulmonary function and neurocognition require further investigation.

TRIAL REGISTRATION

clinicaltrials.gov identifier: NCT00490789

Introduction

Tuberous sclerosis is an autosomal dominant disorder caused by mutations in either *TSC1* or *TSC2*.[1] It is characterised by development of tumours in many organs, including the kidneys and brain and by a range of neuropsychiatric manifestations that include seizures, cognitive impairments and autism.[1] Renal angiomyolipomas affect up to 80% of patients causing morbidity and mortality due to haemorrhage and renal insufficiency. They do not regress spontaneously and current treatments include embolization and nephrectomy.[2,3] Lymphangioleiomyomatosis (LAM) is the main pulmonary manifestation and occurs almost exclusively in females. It can lead to respiratory failure as a consequence of proliferation of smooth-muscle-like cells (LAM cells) and cystic degeneration and may require lung transplantation.[4,5] Lymphangioleiomyomatosis also occurs as a rare sporadic disorder associated with acquired mutations of *TSC2* in women without tuberous sclerosis, forty percent of whom also have renal angiomyolipomas. Identical *TSC2* mutations have been identified in their angiomyolipomas and pulmonary LAM cells indicating a clonal origin and a metastasis-like disease process.[6] The neuropsychiatric manifestations of tuberous sclerosis are ranked by families and carers among the most significant problems.[7] Forty to 50% of patients have intellectual disability, but even those with normal intellectual abilities may have specific neurocognitive problems, including deficits in executive function and memory.[8,9]

Mutation of *TSC1* or *TSC2* leads to over-activation of signaling via the mammalian target of rapamycin complex 1 (mTORC1), a regulator of cell growth.[10] Angiomyolipomas exhibit biallelic *TSC1* or *TSC2* mutation and mTORC1 pathway activation[2]. In the central nervous system mTORC1 regulates neuronal differentiation, synaptic plasticity and the encoding of spatial and auditory memory.[11,12] Sirolimus (rapamycin), a potent mTORC1 inhibitor, reversed renal tumour growth, abnormal synaptic plasticity and deficits in spatial

recall memory in rodent models of tuberous sclerosis.[13-15]. Clinical studies indicate that sirolimus also reduces the size of kidney and brain tumours in patients with tuberous sclerosis.[16-18] In a phase 1-2 trial in twenty patients with tuberous sclerosis or sporadic lymphangioleiomyomatosis, renal angiomyolipoma volumes were reduced by approximately 50% after 12 months of sirolimus treatment but the tumours increased in size when treatment was stopped.[19] In an interim report of the current trial we also showed size reduction of angiomyolipomas in patients who had been treated for up to 12 months.[20] We have now evaluated the longer term efficacy and safety of sirolimus treatment for angiomyolipoma in adults with tuberous sclerosis or sporadic LAM. We also monitored aspects of neurocognitive function and, in those with LAM, assessed changes in lung function.

Methods

Eligibility

Patients with a definite diagnosis of tuberous sclerosis[21] or sporadic lymphangioleiomyomatosis[22] were eligible if they were aged 18 to 65 years, had at least one renal angiomyolipoma of 2 cm or more in longest diameter and an estimated glomerular filtration rate (GFR)[23] of at least 40 milliliters per minute. The major exclusion criteria were: angiomyolipoma embolization within 6 months or bleeding within 12 months, urinary protein excretion of >1 gram per day, inability to walk 100 metres on the flat, requirement for continuous supplemental oxygen or an intelligence quotient (IQ) <70.

Trial Design

This trial was a prospective multicenter phase 2 study (ClinicalTrials.gov number, NCT00490789) conducted at the University Hospital of Wales (UK), Nottingham City Hospital

(UK), Royal Sussex Hospital (UK) and University Hospital Zürich (Switzerland) from October 2005 through September 2009 in accordance with the United Kingdom Medicines for Human Use Regulations 2004, the Declaration of Helsinki, the Good Clinical Practice guidelines of the International Conference of Harmonization and local regulatory requirements. The Thames Valley Multi-Centre Research Ethics Committee approved the protocol and all patients gave written informed consent.

Data Collection

At baseline, angiomyolipomas were visualised by abdominal magnetic resonance imaging (MRI) without contrast media and measured. Up to five angiomyolipomas with longest diameters of 2.0cm or more were selected in each kidney as target lesions and their longest diameters were summated for each patient. Angiomyolipomas with a longest diameter of <2.0cm were recorded as non-target lesions. Oral liquid sirolimus was initiated at a daily dose of 0.5 milligram per m^2 body-surface-area and the dosage was adjusted to achieve steady-state levels between 3 and 6 ng/ml. Follow-up visits were at 3 weeks and at 2, 4, 6, 9, 12, 18 and 24 months after initiation and abdominal MRI was repeated at 2,6,12 and 24 months. If the longest diameter of all target lesions was not reduced by at least 10% at 2 months, the sirolimus dose was increased to achieve a steady-state level of 6 to 10 ng/ml.

At each visit, blood creatinine, lipids, liver enzymes and hematologic parameters were measured, and protein and creatinine were measured in spot urine. GFR was estimated with the MDRD equation[23]. Steady-state levels of sirolimus were determined by liquid chromatography–mass spectrometry from whole blood. Adverse events were classified according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Imaging

Transaxial unenhanced abdominal MRI scans were performed with 1.5 Tesla systems. Scans were acquired with T1-weighted fast spoiled gradient echo and T2-weighted fast spin echo protocols and reviewed by a single radiologist (TD). Cranial MRI was undertaken at baseline and, in patients with tuberous sclerosis, at 12 months using 1.5 Tesla systems. Axial T2 weighted images, axial FLAIR images and coronal 3D SPGR were obtained and reviewed by a single neuroradiologist (JJC). Computed tomography (CT) of the lungs was performed at baseline and at 24 months in patients with lymphangioleiomyomatosis. Images were obtained during full inspiration using a low dose protocol and reviewed by a single radiologist (KP).

Pulmonary Function Testing

Forced Expiratory Volume in 1 second ($FEV_{1)}$, Forced Vital Capacity (FVC) and diffusing capacity of the lung for carbon monoxide (DL_{CO}) were measured at baseline and at 4, 6, 12 and 24 months in a lung function laboratory in all patients with lymphangioleiomyomatosis and expressed as percentage of predicted values[24] to allow for change over time.

Neurocognitive testing

Neurcognitive tests were undertaken at baseline, 4 and 12 months in patients with English as their first language. IQ was determined for eligibility using the National Adult Reading Test.[25] Recall Memory was tested using the List Learning, Story and Complex Figure tests from the Adult Memory and Information Processing Battery (AMIPB)[26]; Recognition

Memory using the Spatial Recognition Memory and Pattern Recognition Memory subtests from the Cambridge Neuropsychological Test Automated Batteries (CANTAB)[27] and Executive Skills using the self-ordered Spatial Working Memory task (SWM), the Stockings of Cambridge (SoC) planning task and the Intra-Dimensional Extra-Dimensional (IDED) set-shifting task, from CANTAB.[27]

Statistical Analysis

The primary outcome was the number of patients in whom renal angiomyolipomas responded to sirolimus therapy. Response was evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST)[28] that define a "complete response" as the disappearance of all lesions, a "partial response" as decrease of at least 30% in the sum of the longest diameters of target lesions without progression of non-target lesions, "progressive disease" as a 20% increase in the sum of the longest diameters of target lesions compared with the smallest value recorded since treatment started or progression of non-target lesions or appearance of 1 or more new lesions and "stable disease" as neither response nor progression. In this trial a response was defined as a complete or partial response that occurred at any time during the trial.

We aimed initially to recruit 15 patients with sporadic lymphangioleiomyomatosis and 15 with tuberous sclerosis but because of slow recruitment we modified our plan to recruiting at least 14 patients in total. The minimum sample size of 14 was based on Fleming's single stage design.[29] p1 (the response rate below which the treatment would not be studied further) was set at 0.1 and p2 (the response rate above which the treatment would be studied further) at 0.4. We set α (the probability of concluding that the response rate is greater than p1 when that is false) at 0.05 and β (the probability of concluding that the response rate is less than p2 when that is false) at 0.1. The primary analysis was based upon all patients.

The per protocol population included all patients in the study apart from those who received less than four months treatment at the higher sirolimus blood level of 6-10ng/ml, unless a response had been achieved.

Annual rate of change in FEV_1, FVC and DL_{CO} was determined by linear regression.

For neurocognitive outcomes, we used published UK percentile bands for age and sex for each test to derive summary scores[30] for each neurocognitive domain i.e. recall memory, recognition memory and executive function.

Results

Patients and Protocol Completion

Sixteen patients, 13 female and 3 male, were enrolled. Ten had tuberous sclerosis (including 3 with LAM) and 6 had sporadic lymphangioleiomyomatosis with no skin signs of tuberous sclerosis or manifestations of tuberous sclerosis on brain MRI scan. Four had undergone previous unilateral nephrectomy. Among the 12 patients with two kidneys the target angiomyolipomas (i.e. those of 2 cm or more in longest diameter) were bilateral in 6. During the trial one patient with severe sporadic lymphangioleiomyomatosis died from a respiratory infection, one withdrew for lung transplantation, one for elective surgery for angiomyolipoma, two because of protocol deviations and one because of likely sirolimus-related peripheral edema. Ten patients completed the 24 month trial (Figure 1). At 2 months, 4 patients were dose escalated to steady-state blood levels of 6-10 ng/ml. Others were maintained at 3-6 ng/ml without further dose escalation.

Response of angiomyolipomas

The overall response rate by the RECIST criteria[28] was 50% (8 of 16) and in the per protocol group it was 80% (8 of 10). All were partial responses. They occurred in 4 of 10 patients with tuberous sclerosis and 4 of 6 with sporadic lymphangioleiomyomatosis. At 24 months a partial response was present in 4 of 10 patients (40%) remaining in the trial. Moreover, in every patient the sum of the longest diameters of target angiomyolipomas was reduced throughout the trial compared to baseline (Figure 2 and Online Supplementary Table 1). Of 23 target angiomyolipomas evaluated at 24 months, 21 were smaller and 2 were unchanged (Table 1). Of all 48 target angiomyolipomas evaluated during the trial 41 (85%) were smaller at the last measurement than at baseline, 2 were unchanged and 5 were larger (including 4 from one patient, TSC4, and a 1mm increase in the fifth). (Table 1) Angiomyolipomas shrank most quickly early in the trial (Figure 2). For angiomyolipomas measured at baseline and at 12 and 24 months there was a mean reduction in longest diameter of 7.3mm at 12 months but a further mean reduction of only 0.7 mm at 24 months (Table 1).

Lung Function

Lung function in patients with sporadic and tuberous sclerosis-associated lymphangioleiomyomatosis showed wide variation at baseline and fell slightly in most patients during the trial (Figure 3 and Online Supplementary Table 2). For the 5 patients with measurements over 2 years the mean (+/- SD) FEV_1 declined by 76 +/-52 ml/yr, mean FVC by 55 +/-94 ml/yr and mean DL_{co} by -0.49 +/- 0.55 ml/min/mmHg/yr.

Four patients (LAM1, LAM2, LAM4 and TSC1) had serial measurements of FEV_1 prior to enrollment, for 16, 132, 42 and 106 months respectively. Mean annual change in FEV_1 for these patients before and during the trial were -172 and -94, -122 and -89, -90 and +10 and -49 and -69 ml/yr respectively. There were no clinically relevant changes in lung CT

appearances during the trial.

Neurocognitive function

The mean (+/-SD) IQ of patients with tuberous sclerosis was 107 (+/-12) and of sporadic lymphangioleiomyomatosis patients 105 (+/-15). Four patients with tuberous sclerosis were taking antiepileptic drugs during the trial but none reported seizures during the trial or in the preceding year. Baseline cranial MRI scans in patients with tuberous sclerosis showed cortical tubers and sub-ependymal nodules but no subependymal giant cell astrocytomas and no changes were seen at 12 months.

Increased recall memory scores were seen in 7 of the 8 tested patients with tuberous sclerosis. By contrast, recognition memory scores fell in 5 and none showed an increase. Executive skill scores increased in 5 of 8 patients with tuberous sclerosis. Changes in neurocognitive scores were also observed in patients with sporadic lymphangioleiomyomatosis (Figure 4 and Online Supplementary Table 3).

Adverse events

The most common adverse events were oral mucositis (6 of 16 patients), respiratory infections (5 patients) and proteinuria (5 patients) (Table 2). Seven serious adverse events occurred of which 3 were possibly related to sirolimus. One patient with severe sporadic lymphangioleiomyomatosis died following a respiratory infection and two further patients with lymphangioleiomyomatosis were hospitalized, one with pharyngitis and one with a chest infection. The four remaining serious adverse events were categorized as not related or unlikely to be related to sirolimus: a fractured tibia and fibula, chest pain of unknown cause, urinary obstruction secondary to an ovarian cyst and musculoskeletal back pain.

Discussion

This trial determined the response of renal angiomyolipomas in patients with tuberous sclerosis or sporadic lymphangioleiomyomatosis to 2 years of sirolimus treatment. A tumour response by RECIST criteria[28] was observed 8 of 16 patients overall and 8 of 10 in the per-protocol group. Of 23 angiomyolipomas evaluated at 24 months, 21 were smaller and 2 were unchanged. Although all target angiomyolipomas shrank in most patients, one (TSC4) who had the most target lesions and who withdrew at 12 months, showed heterogeneity of tumour response with 5 of 9 angiomyolipomas shrinking and 4 growing. One previous trial reported by Bissler et al. investigated sirolimus treatment for angiomyolipomas in patients with tuberous sclerosis or sporadic lymphangioleiomyomatosis and found a mean reduction in angiomyolipoma volume of 47% at 12 months.[19] In the current trial angiomyolipomas measured at 12 months showed a mean reduction in their longest diameters of 25% compared to baseline, equivalent to a volume reduction of 60% if a spherical shape is assumed. While 12 of 16 patients in the current trial were maintained at trough blood levels of 3-6 ng/ml and the others 4 at 6-10 ng/ml all but one of the patients in the trial by Bissler et al. were escalated to trough blood levels of 10-15 ng/ml. The current trial shows that sirolimus levels at the lower end of the immunosuppressive range are effective in reducing angiomyolipoma size in tuberous sclerosis or sporadic lymphangioleiomyomatosis.

In the trial reported by Bissler et al. angiomyolipoma volumes increased after sirolimus therapy was withdrawn at 12 months, returning from 53% to 85.9% of the baseline value by 24 months.[19] In the current trial the mean longest diameter of angiomyolipomas measured at both

12 and 24 months was 2.19cm and 2.11cm respectively, indicating that tumour response was maintained by continuation of therapy but little further shrinkage occurred during the second year of treatment. Long term sirolimus therapy may be needed to prevent tumour re-growth in patients with tuberous sclerosis or lymphangioleiomyomatosis and this could be acceptable, as it is in organ transplant recipients.

In recent studies of lymphangioleiomyomatosis the mean rate of decline in FEV_1 has ranged from 75-118 ml/yr.[31-33] During this trial it was 76 ml/yr in the 5 patients who had measurements over 2 years and 49 ml/yr in 7 patients with measurements over one year or more. We did not observe a significant improvement in lung function during sirolimus therapy as was reported by Bissler et al.[19] but neither study was powered to evaluate lung function. A recently reported randomised control trial of sirolimus for the treatment of lymphangioleiomyomatosis suggests that sirolimus may prevent or reduce decline in lung function, consistent with our findings[34].

The reversal of spatial learning deficits by rapamycin (sirolimus) treatment in heterozygous Tsc2 mice[15] has suggested that mTORC1 inhibitors might also improve specific neurocognitive problems associated with tuberous sclerosis.[35] The non-randomised design and small patient numbers restricted the analysis of neurocognitive outcomes in this trial and the patients with tuberous sclerosis were mildly affected with far fewer neurocognitive problems than more typical patients. Nevertheless, scores for recall memory, that has been associated with mTOR activity[15], increased in 7 of 8 patients with tuberous sclerosis while those for recognition memory did not. Larger randomised control trials are warranted to determine whether treatment-related changes in neurocognition occur with mTORC1 inhibition in patients with and without tuberous sclerosis.

Adverse events were common, consistent with the known toxicities of sirolimus and mostly of low grade. However, one patient with severe lymphangioleiomyomatosis died of a respiratory infection during this study. Because of their immunosuppressive properties, the risks and benefits of mTORC1 inhibitors need specific and careful evaluation in this setting.

Although our trial involved a small number of patients and was non-controlled and open label it reports one of the earliest examples of therapeutic targeting of tumours in the context of both a mendelian and a sporadic disorder by inhibition of a shared signaling pathway. The high response rate seen in the trial underscores the potential for effective targeted treatment when the setting is one of relative molecular homogeneity.

Recruitment to the trial proved difficult, reflecting the rarity of these disorders and our restrictive inclusion criteria, particularly for patients with tuberous sclerosis. Nonetheless, it provides evidence that renal angiomyolipomas in patients with tuberous sclerosis or sporadic lymphangioleiomyomatosis decrease in size in response to treatment with sirolimus and that this response is maintained by continuation of treatment for 2 years. Larger trials should now address whether, in patients with large tumours and/or extensive renal involvement, mTORC1 inhibition leads to reduction of the clinically important complications of renal angiomyolipoma, notably haemorrhage and renal failure.

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of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to writing this report and vouch for its fidelity to the trial protocol and statistical analysis plan.

Figure Legends

Figure 1. **Flow diagram summarising patients and assessments.**

One patient who was still in the study was unable to undergo magnetic resonance imaging of the kidneys at the 2-month assessment and another at the 6-month assessment because of inter-current illness. [#] One patient was unable to undergo pulmonary function tests at 6 and at 12 months because of inter-current illness.

Figure 2. **Changes in angiomyolipoma burden during sirolimus therapy.**

Renal angiomyolipomas were measured at baseline and at 2,6,12 and 24 months by magnetic resonance imaging. The sum of the longest diameters of all target angiomyolipomas in each patient was calculated at each time point and the percentage reduction calculated by comparison to the baseline value. Each solid line shows change in angiomyolipoma burden in one patient. The upper dashed line represents the baseline value and the lower dashed line 70% of the baseline value. Patients in whom the sum of the longest diameters of target angiomolipomas fell below 70% of the baseline value are defined as partial responders by the RECIST criteria.

Figure 3. **Pulmonary function studies in patients with sporadic or tuberous sclerosis-associated lymphangioleiomyomatosis.**

Forced expiratory volume at 1 second (FEV1), forced vital capacity (FVC) and diffusing capacity of the lung for carbon monoxide (DL_{co}) are shown as percentages of the predicted value for women of equivalent age and height[24].

Figure 4. **Changes in Neurocognitive Test Performance in Patients with Tuberous Sclerosis (TSC) and Sporadic Lymphangioleiomyomatosis (LAM).**

Neurocognitive test performance at baseline and at 12 months (but at 4 months in patients TSC4 and LAM6 who withdrew prior to the 12 month assessment) was measured using parallel test versions. Test scores were converted to percentile band scores according to published UK norms for age and sex for each test version. This enabled comparisons to be made between parallel test versions. We calculated summary scores across the tests in each neurocognitive domain[30] by allocating an integer score to each centile band (below 5^{th} = 1, 5^{th}-9^{th} = 2, 10^{th}-24^{th} = 3, 25^{th}-49^{th} = 4, 50^{th}-74^{th} = 5, 75^{th}-89^{th} = 6, 90^{th} and above = 7) and adding the integer scores (given in Supplementary Table 3) together. (A) The total immediate recall memory score was the sum of the integer scores for list leaning, story recall and figure recall; (B) the total immediate recognition memory score was the sum of the pattern recognition and spatial recognition integer scores and (C) the total executive score was the sum of the SWM, SOC and IDED integer scores.

Tables

Patient	Angiomyolipoma	baseline	2 Months	6 Months	12 Months	24 Months
LAM1	R1	2.2	1.4	ND	2.0	Deceased
	R2	2.6	2.7	ND	2.3	Deceased
	R3	2.3	2.3	ND	1.9	
LAM2	R1	2.8	2.5	1.4	1.2	1.0
	R2	2.0	2.0	1.9	1.6	1.4
LAM3	R1	2.2	1.9	1.9	1.9	1.3
LAM4*	L1	2.8	2.5	2.8	2.4	2.2
	L2	2.0	1.9	1.6	1.3	0.8
LAM5	R1	2.3	2.2	Withdrew		
LAM6	L1	5.6	3.4	Withdrew		
	L2	2.7	1.7	Withdrew		
	L3	2.6	1.8	Withdrew		
TSC1(LAM)	R1	2.4	1.9	1.9	1.9	2.0
	R2	2.2	1.4	1.4	1.3	1.3
TSC2(LAM)	R1	3.2	2.6	2.1	2.3	Withdrew
	R2	2.2	1.8	1.4	1.6	Withdrew
	R3	2.2	2.1	2.0	2.0	Withdrew
	L1	3.8	3.4	2.8	2.4	Withdrew
TSC3	R1	2.4	2.2	2.1	Withdrew	
	R2	2.4	1.9	1.9	Withdrew	
	L1	5.4	4.6	4.3	Withdrew	
	L2	2.2	2.4	1.9	Withdrew	
	L3	2.7	2.6	2.8	Withdrew	
TSC4	R1	14.1	12.8	13.3	Withdrew	
	R2	2.7	2.7	2.5	Withdrew	
	R3	3.4	3.3	3.7	Withdrew	
	R4	3.2	4.2	4.2	Withdrew	
	R5	5.4	5.4	6.4	Withdrew	
	L1	10.6	10.2	8.3	Withdrew	
	L2	4.4	4.5	4.8	Withdrew	
	L3	3.6	3.5	3.4	Withdrew	
	L4	3.4	3.3	3.2	Withdrew	
TSC5*	R1	2.3	1.9	1.8	1.8	1.6
	R2	4.5	3.8	2.8	2.8	2.9
TSC6	R1	2.4	ND	2.3	1.8	1.6
	L1	2.8	ND	2.3	1.7	2.1
	L2	2.4	ND	2.6	1.9	1.9
TSC7*	R1	2.5	2.4	2.2	1.8	1.8
	R2	3.8	2.8	2.7	2.8	3.2
	R3	3.3	2.4	2.3	2.1	2.1
	R4	2.9	2.2	2.4	2.1	1.9

Patient	Angiomyolipoma	baseline	2 Months	6 Months	12 Months	24 Months
TSC8(LAM)	R1	3.9	2.3	2.6	2.5	2.5
	L1	4.3	3.1	3.6	4.2	4.3
TSC9*	R1	2.0	1.7	1.7	1.9	1.7
	R2	4.2	3.5	3.8	3.2	3.2
TSC10	R1	2.9	2.8	2.8	2.2	2.0
	L1	3.2	2.9	2.7	2.5	2.5
	L2	3.3	3.5	3.3	3.4	3.3

Table 1. **Longest Diameters of Individual Target Angiomyolipomas**.

Target angiomyolipomas in the right (R) and/or left (L) kidney in each patient were visualised by magnetic resonance imaging (MRI) at baseline and at 2,6,12 and 24 months and the longest diameter of each angiomyolipoma was measured. Values are in cm. ND = MRI not done due to inter-current illness. TSC1-TSC10 = patients with tuberous sclerosis. LAM1-LAM6 = patients with sporadic lymphangioleiomyomatosis. TSC(LAM) indicates patients with tuberous sclerosis and lymphangioleiomyomatosis. * These patients had previous unilateral nephrectomy for angiomyolipoma.

Category	Diagnosis	No. of Events	No. of patients	Grade 1-2	Grade 3-4	Grade 5
GASTROINTESTINAL						
Oral mucositis	All	9	**6**	9	-	-
	SLAM	4	**3**	4	-	-
	TLAM	3	**2**	3	-	-
	TSC	2	**1**	2	-	-
Diarrhoea	All	1	1	1	-	-
	SLAM	1	1	1	-	-
	TLAM	-	-	-	-	-
	TSC	-	-	-	-	-
Nausea	All	1	1	1	-	-
	SLAM	1	1	1	-	-
	TLAM	-	-	-	-	-
	TSC	-	-	-	-	
INFECTION						
Upper respiratory tract or bronchitis	All	16	5	14	1	1
	SLAM	16	5	14	1*	1*
	TLAM	-	-	-	-	-
	TSC	-	-	-	-	-
Pharyngitis	All	1	1	-	1	-
	SLAM	1	1	-	1*	-
	TLAM	-	-	-	-	-
	TSC	-	-	-	-	-
Urinary tract infection	All	3	3	3	-	-
	SLAM	2	2	2	-	-
	TLAM	1	1	1	-	-
	TSC	-	-	-	-	-
Cellulitis	All	2	2	2		
	SLAM	-	-	-	-	-
	TLAM	1	1	1	-	-
	TSC	1	1	1	-	-
Oral cavity	All	1	1	1	-	-
	SLAM	1	1	1	-	-
	TLAM	-	-	-	-	-
	TSC	-	-	-	-	-
METABOLIC						
Proteinuria	All	5	5	5	-	-
	SLAM				-	-
	TLAM	1	1	1	-	-
	TSC	4	4	4	-	-
Raised creatine kinase	All	3	3	3	-	-
	SLAM				-	-
	TLAM	2	2	2	-	-
	TSC	1	1	1	-	-
Hypertriglyceridaemia	All	2	2	2	-	-
	SLAM	-	-	-	-	-
	TLAM	1	1	1	-	-
	TSC	1	1	1	-	-
Raised ALP	All	1	1	1	-	-
	SLAM	-	-	-	-	-
	TLAM	-	-	-	-	-
	TSC	1	1	1	-	-
Hypokalaemia	All	1	1	1	-	-
	SLAM	1	1	1	-	-
	TLAM	-	-	-	-	-
	TSC	-	-	-	-	-
SOFT TISSUES						
Peripheral oedema	All	3	3	3	-	-
	SLAM	-	-	-	-	-
	TLAM	1	1	1	-	-
	TSC	2	2	2	-	-

Category	Diagnosis	No. of events	No. of patients	Grade 1-2	Grade 3-4	Grade 5
CONSTITUTIONAL SYMPTOMS						
General malaise	All	1	1	1	-	-
	SLAM	1	1	1	-	-
	TLAM	-	-	-	-	-
	TSC	-	-	-	-	-
Fatigue	All	3	3	3	-	-
	SLAM	-	-	-	-	-
	TLAM	2	2	2	-	-
	TSC	1	1	1	-	-
DERMATOLOGY						
Acneform rash	All	2	2	2	-	-
	SLAM	-	-	-	-	-
	TLAM	-	-	-	-	-
	TSC	2	2	2	-	-
Exacerbation of eczema	All	1	1	1	-	-
	SLAM	-	-	-	-	-
	TLAM	1	1	1	-	-
	TSC	-	-	-	-	-
CARDIAC						
Palpitations	All	2	2	2	-	-
	SLAM	2	2	2	-	-
	TLAM	-	-	-	-	-
	TSC	-	-	-	-	-
NEUROLOGY						
Depression	All	1	1	1	-	-
	SLAM	-	-	-	-	-
	TLAM	-	-	-	-	-
	TSC	1	1	1	-	-
ENDOCRINE						
Hypothyroidism	All	1	1	1	-	-
	SLAM	-	-	-	-	-
	TLAM	-	-	-	-	-
	TSC	1	1	1	-	-
OCULAR						
Retinal tear	All	1	1	1	-	-
	SLAM	-	-	-	-	-
	TLAM	1	1	1	-	-
	TSC	-	-	-	-	-
BLOOD						
Anaemia	All	1	1	1	-	-
	SLAM	-	-	-	-	-
	TLAM	-	-	-	-	-
	TSC	1	1	1	-	-

Table 2. **Sirolimus-Related Adverse Reactions.**

Sirolimus related adverse reactions were those adverse events that were considered to be possibly, probably or definitely related to sirolimus. *These events were reported as severe adverse events, defined as those that resulted in death or were life-threatening, required hospitalization or resulted in disability. “All”, All patients N = 16; “SLAM”, Sporadic lymphangioleiomyomatosis N = 6; “TLAM”, tuberous sclerosis with lymphangioleiomyomatosis N = 3; “TSC”, tuberous sclerosis only N = 7.

Online-Only Material: Supplementary online materials are Tables 1-3.

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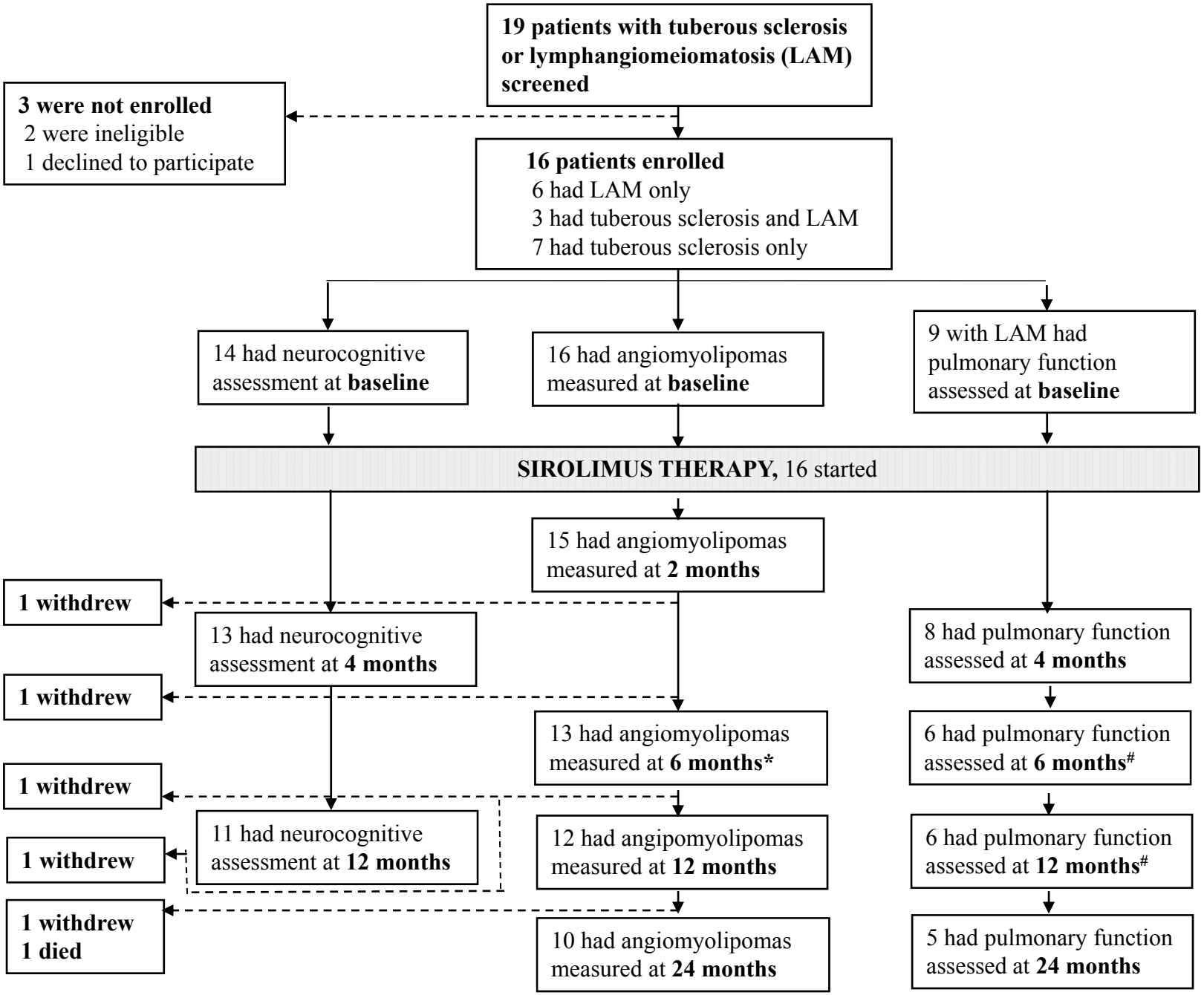
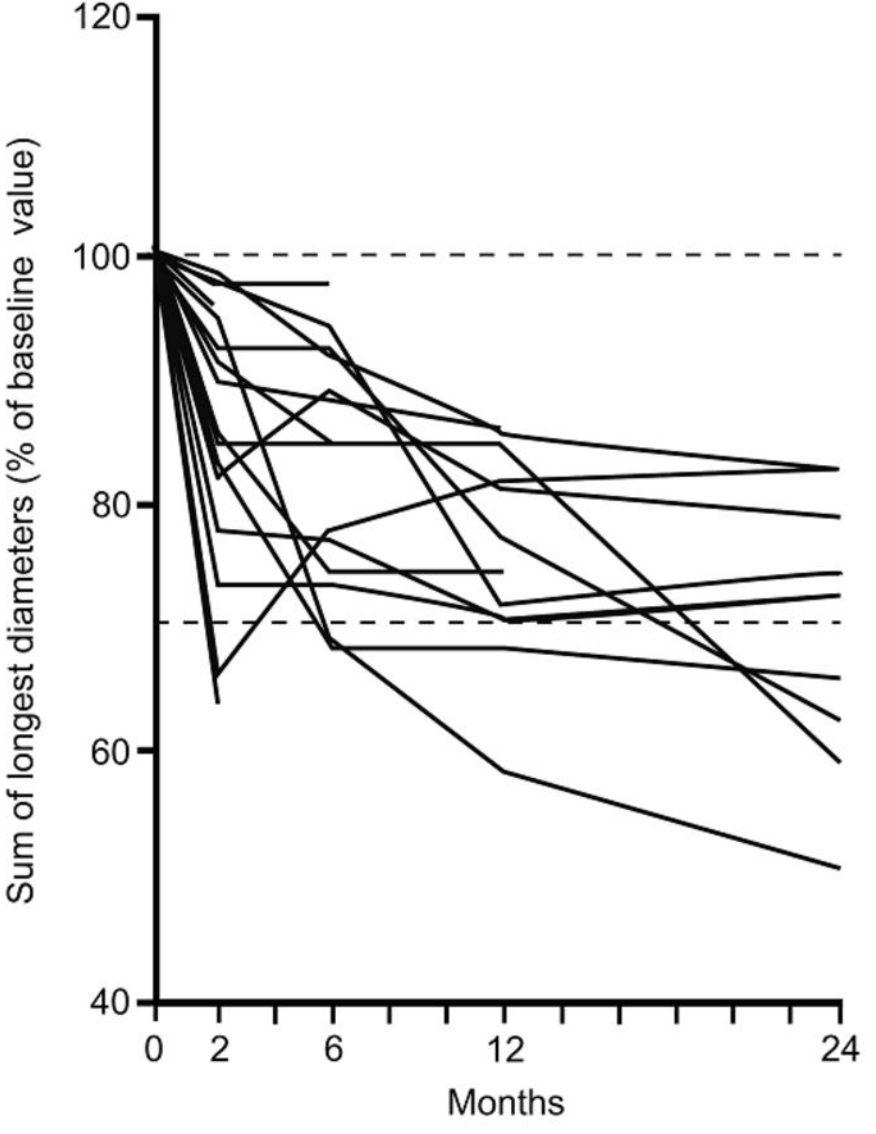
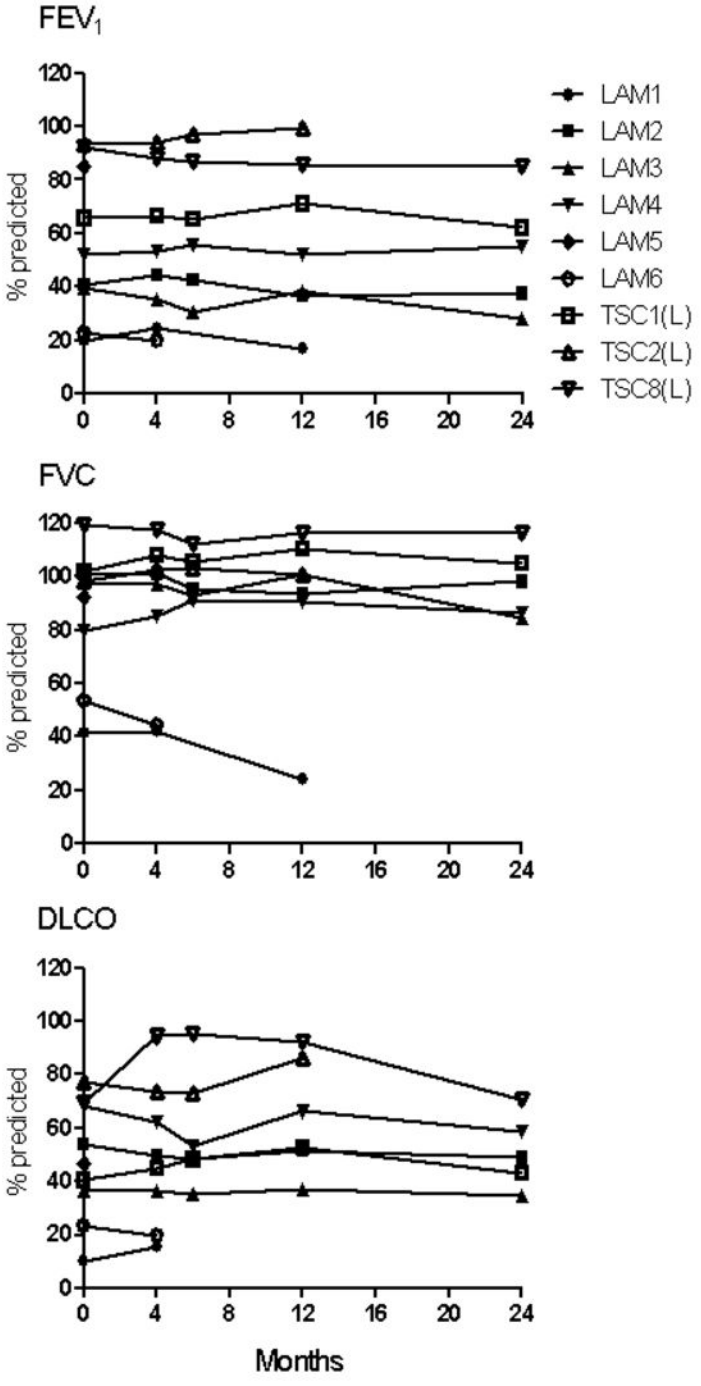
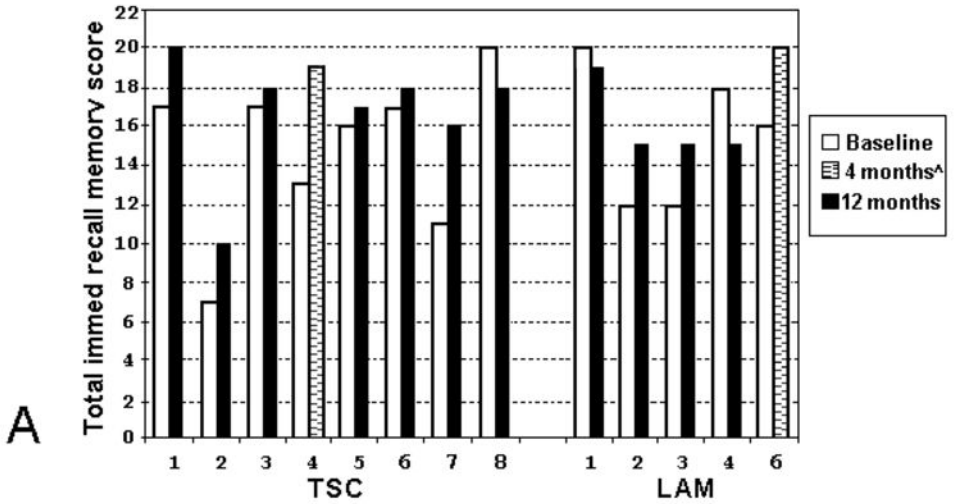
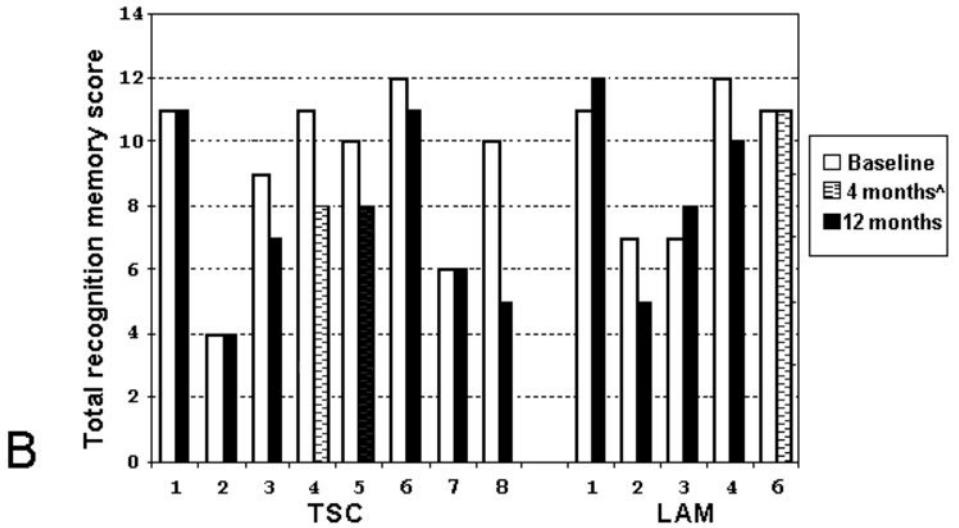
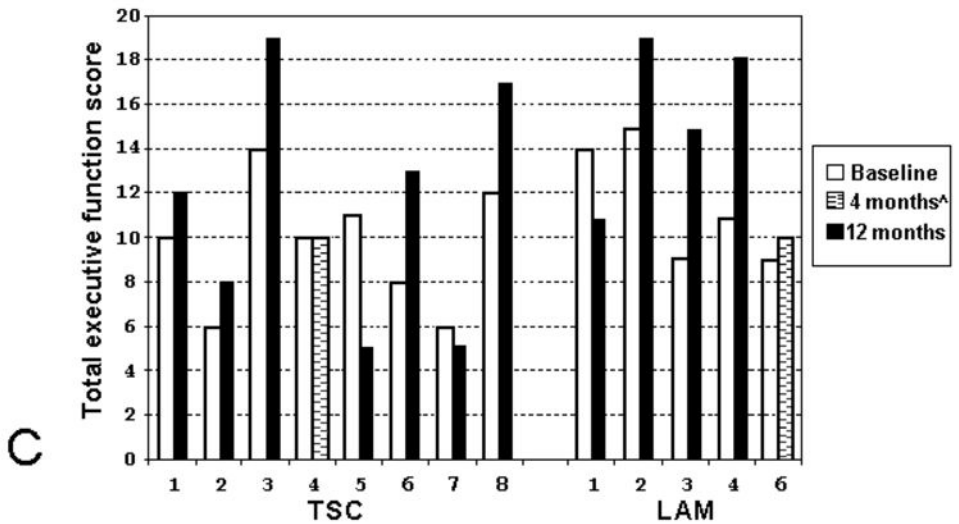

19 patients with tuberous sclerosis or lymphangiomeiomatosis (LAM) screened
3 were not enrolled
2 were ineligible
1 declined to participate
16 patients enrolled
6 had LAM only
3 had tuberous sclerosis and LAM
7 had tuberous sclerosis only
14 had neurocognitive assessment at baseline
16 had angiomyolipomas measured at baseline
9 with LAM had pulmonary function assessed at baseline
SIROLIMUS THERAPY, 16 started
15 had angiomyolipomas measured at 2 months
1 withdrew
13 had neurocognitive assessment at 4 months
8 had pulmonary function assessed at 4 months
1 withdrew
13 had angiomyolipomas measured at 6 months*
6 had pulmonary function assessed at 6 months#
1 withdrew
1 withdrew
11 had neurocognitive assessment at 12 months
12 had angipomyolipomas measured at 12 months
6 had pulmonary function assessed at 12 months#
1 withdrew
1 died
10 had angiomyolipomas measured at 24 months
5 had pulmonary function assessed at 24 months


120
100
80
60
40
Sum of longest diameters (% of baseline value)
0
2
6
12
24
Months


FEV1
% predicted
120
100
80
60
40
20
0
0
4
8
12
16
20
24
LAM1
LAM2
LAM3
LAM4
LAM5
LAM6
TSC1(L)
TSC2(L)
TSC8(L)
FVC
% predicted
DLCO
% predicted
Months


A
Total immed recall memory score
22
20
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8
TSC
1 2 3 4 6
LAM
Baseline
4 months^
12 months


B
Total recognition memory score
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8
TSC
1 2 3 4 6
LAM
Baseline
4 months^
12 months


C
Total executive function score
20
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8
TSC
1 2 3 4 6
LAM
Baseline
4 months^
12 months